

# **TDM Roundtable Recommendations: Lamotrigine Assay Validation Guidance**

## **TDM Roundtable Participating Organizations:**

American Association for Clinical Chemistry  
American Society for Clinical Laboratory Science  
American Society for Clinical Pathology  
College of American Pathologists  
Food and Drug Administration

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*C. E. Pippenger, PhD, Co- chair*

*Dave Berry, PhD, Co-chair*

*Richard Earley, PhD*

*Christine Goodall*

*Robert Murray, JD, PhD*

*Elvan Sahin, PhD*

*Steven Wong, PhD*

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# **TDM Roundtable Recommendations: Lamotrigine Assay Validation Guidance**

## **1. Introduction**

This guidance document is intended to serve as a set of recommendations to researchers and manufacturers to facilitate the development and validation of therapeutic drug management (TDM) assays for lamotrigine. Although the phrase “therapeutic drug monitoring (TDM)” has been used for many years and in many places to refer to quantitative measurement of therapeutic drugs in serum or plasma in order to assist a care provider to ensure that a patient is treated with optimal concentration of the drug in question, we have replaced “management” for “monitoring” in order to emphasize the purpose of the testing. “Management” implies that the laboratory measurement is an essential part of the treatment of the patient, whereas “monitoring” is focused on the analytical process, without reference to the clinical implications. The abbreviation “TDM” is retained throughout this document, but it is intended to refer to “management” and not to “monitoring.” As such it will establish scientifically sound expectations which are useful for documenting analytical performance of new testing devices or methods for lamotrigine.

The remaining sections of this document describe the information generally needed in an FDA application for a TDM assay. Specific information related to lamotrigine TDM assays is provided in the Annexes. Before undertaking development of any new TDM assay, the manufacture is strongly encouraged to contact the FDA, Office of In Vitro Diagnostics to discuss their validation strategy for FDA clearance or approval.

## **2. Background**

Therapeutic Drug Management (TDM) assays are quantitative measures of a specific drug concentration in plasma or serum, and serve to aid in the management of a patient’s drug therapy. As analytical techniques, they are expected to accurately measure the concentration of the target drug, with defined precision, sensitivity, and specificity. While the typical specimen is plasma or serum, it is possible to validate the assay to be used to test drug concentration in other biological samples e.g. whole blood, saliva, urine, or milk. Typically the metabolism and pharmacokinetics of the drug which is the subject of the proposed assay will have been established and published in the scientific literature before a TDM assay is developed. The pharmacokinetics information, for the various matrices for which the test is intended and biological variations thereon, should be included in the information submitted to FDA, and in the information (package insert) provided to the user of the assay. Typically there is also information in the literature regarding optimal ranges. This information should also be presented.

### **2.1. Therapeutic Drug Management of Antiepileptic Drugs (AEDs)**

Since seizures occur at irregular intervals, pharmacological therapy is often empiric and prophylactic in nature. In addition, occurrence of adverse effects may be insidious, and knowledge of an upper

concentration limit can be useful in avoiding treatment-emergent adverse effects. Specifically, TDM can be useful in establishing an individual patient's optimal serum/plasma concentration range, and benchmarking serum concentrations at which seizures are controlled, as well as those associated with AED-specific adverse effects. TDM can also assist with management whenever a patient's medication regimen is changed (e.g. addition or removal of potentially interacting concomitant medications), or when physiologic changes occur (e.g. co-morbid hepatic or renal disease, pregnancy and normal physiological changes such as puberty and aging). TDM results in more efficient and effective optimization of therapy and patient management. AEDs that display significant pharmacokinetic interpatient variability, or are subject to multiple drug/drug interactions are likely to benefit most from TDM.

For Therapeutic Drug Management to be useful it is important that serum sampling is conducted in a consistent manner. With patients receiving chronic therapy this would include, whenever possible, obtaining steady-state, trough (pre-dose) blood samples. These should be submitted to the laboratory with an assessment of recent drug intake (e.g. medication dose administered, time of last dose, patient age, weight and height ).

## **2.2. Metabolism and disposition**

To date no commercially available immunoassay has been developed for lamotrigine and the majority of data regarding drug levels has been derived using chromatographic techniques (Berry, 1992; Lensmeyer, 1997). An optimal trough serum/plasma concentration range for lamotrigine is 3-15 mg/L (Johannessen, 2003.). Lamotrigine is frequently prescribed as one component of a multiple AED treatment and is subject to many significant pharmacokinetic drug interactions. Some concomitantly administered drugs such as sodium valproate can strongly inhibit the metabolism of lamotrigine (approximately 50-60%), resulting in a marked increase in plasma/serum lamotrigine concentrations (Anderson, 1996; Gidal, 2000; Gidal, 2001; Sale, 2002; Yuen, 1992). Importantly, this medication combination has been associated with an increased risk of potentially severe dermatological reactions including Stevens-Johnson syndrome. Conversely, other enzyme inducing AEDs such as phenytoin, carbamazepine, Phenobarbital, and/or other concomitantly administered drugs which are also enzyme inducers can significantly increase lamotrigine clearance by up to 75-100% resulting in a marked decrease in serum concentrations (May, 1996; Anderson, 2002). Furthermore, large interpatient variability has been observed in lamotrigine concentrations in patients on monotherapy, indicating that large pharmacokinetic variations may exist between individuals thus endorsing the value of TDM for optimizing therapy (Werz, 1999). Appendix 1 summarizes the information that is available concerning the metabolism and disposition of lamotrigine. In addition, Appendix 1 indicates that one of the factors affecting lamotrigine concentration is pharmacogenomics.

## **3. Risks to Health**

There are no known *direct* risks to patient health associated with lamotrigine assay. Potential *indirect* risks exist, associated with the clinical consequences of an erroneous TDM result, which may cause inappropriate patient dosing, leading to an inadequate or ineffective drug level in the patient, or to a toxic concentration.

Risks to health generally associated with the use of the lamotrigine assays are given in Appendix 2 together with the measures recommended to mitigate these identified risks. A risk analysis should also be conducted to identify any other risks specific to the testing device under development and the risk analysis method should also be described. If an alternative approach is used to address a particular risk identified in this guidance document, or if risks are identified in addition to those in the guidance, then sufficient detail to support the approach used to address the risks should be provided. It would also be helpful to consult with FDA early in the assay development process concerning study requirements in such cases. If there are other patient management risks, these should be addressed in the product labeling.

## **4. Performance Characteristics**

### **a. General Study Recommendations**

Patient samples or sample pools, derived from the intended use population (i.e., patients being treated with the drug in question) should be included in the analytical protocols described below. Spiked samples may be used under some circumstances, but at a minimum, samples from patients taking the target drug, must be included in the precision and recovery studies, as well as method comparison studies. This is important because patient samples reflect the relevant proportions of free and bound drug, metabolites, and other drugs commonly co-administered to the type of patients who require the target drug; therefore this is essential to demonstrate the robustness of the assay.

Spiked samples can be used to supplement the studies; however caution must be exercised against using spiked samples as the only matrix in the evaluations, because spiked samples, which may or may not contain metabolites of the target drug, provide a less complete assessment of the performance characteristics.

The effect of freezing/thawing samples, variables in collection and storages, should also be thoroughly investigated

All analytical protocols should be performed according to the procedures specified by the manufacturer in the testing program. The package insert will subsequently be developed from the studies and will reflect the level of performance that can be achieved when the assay is performed according to the package insert. Therefore, each pre-analytical and analytical step must be specified and included in each of the analytical studies; pre-analytical pretreatment steps, for example, should be included for individual replicates in a precision study and for individual dilutions in a linearity study. All of the manufacturer's recommended quality control and calibration procedures must be followed.

Appropriate specifics concerning protocols should be provided so that results can be interpreted properly and duplicated, if necessary. These specifics are also necessary to aid users in interpreting information in the labeling. For example, when referring to National Committee for Clinical Laboratory Standards (NCCLS) evaluation protocols or guidelines, indicate which specific aspect of the protocols or guidelines were followed.

In studies using spiked samples, information should be provided to document the purity of drugs, metabolites, or potential interferents, as well as the type of sample that the drug is spiked into.

Serum/plasma is the matrix recommended for most TDM assays, and equivalence must be demonstrated using the commonly employed anticoagulants and collection devices. In cases where whole blood or other biological matrices are to be analyzed, this should be clearly stated and appropriate correlations (comparison to serum or plasma assays, and comparisons among different anticoagulants) must be provided.

#### **b. Specific Performance Characteristics**

The following performance characteristics should be assessed in order to document performance and properly label the device in conformance with 21 CFR 809.10(b)(12).

##### **(1) Precision**

Within-run, and total precision should be characterized according to guidelines provided in "Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline" (1999) National Committee for Clinical Laboratory Standards (NCCLS), Document EP05-A<sup>2</sup>. That document includes guidelines for experimental design, computations, and format for statement of claims.

For lamotrigine the precision of the assay should be evaluated for at least three concentrations spanning most of the assay range. Typically these concentrations are chosen to represent (a) sub-optimal range or near low end of the reportable range (b) concentrations considered to be within the optimal range and (c) near high end of reportable range or toxic range.

Whenever possible, precision studies should be performed utilizing patient specimens. If patient specimens are not readily available at the time initial precision studies are performed then spiked serum/plasma samples may be used, but as soon as possible during assay development, precision utilizing patient specimens should be evaluated to confirm that other compounds present in the patients' biological fluids do not affect the TDM assay precision. When interpreting the significance of precision values it is important to recognize that the smallest coefficient of variation is the goal. However, it is equally important to recognize that clinical decision points associated with the interpretation of TDM values are generally reflected by a 20% change. For lamotrigine the between batch precision goal is 10% or better.

The description of the protocol and results should include the items listed below:

- sample types (e.g., pooled patient samples, spiked serum/plasma)
- point estimates of the concentration
- standard deviations of within-run and total precision
- sites at which precision protocol was run

- number of days, runs, and observations.
- calibration curve stability (if stored)

The factors that were held constant and which were varied during the evaluation (e.g., instrument calibration, reagent lots, and operators) should also be identified. Computational methods, if they differ from those described in NCCLS EP05-A, should also be identified.

## **(2) Recovery**

As a measure of accuracy, the percent recovery of lamotrigine should be characterised. Typically, these studies involve spiking known amounts of pure lamotrigine into samples that are either negative for this drug or from patients taking lamotrigine that contain known drug concentrations. Spiking into samples from patients taking lamotrigine should be included as part of the study. Final concentrations of the spiked samples should span a significant part of the reportable range and include potential medical decision levels.

Recovery should be determined at both sub optimal and toxic concentrations to verify consistent performance across the assay range.

Replicates of each concentration or sample should be evaluated and the number of replicates chosen to ensure that any clinically significant differences observed will be statistically significant. Description of the study protocol should include:

- sample types and concentrations
- statement of how target concentrations were determined
- materials used for spiking
- number of replicates
- definition or method of calculating recovery.

When reporting results, the range of recoveries for each concentration evaluated should be indicated since this approach is more informative than describing mean recoveries at each concentration level.

## **(3) Linearity**

For lamotrigine the linear range of the assay response should be 1-30 mg/L and should be characterized by evaluating samples whose lamotrigine concentrations are known relative to one another. When practical, the linearity of the assay should be characterized using dilutions of patient samples containing elevated lamotrigine

concentrations. Spiked serum/plasma may be used when patient samples are not available, (for example at very high drug concentrations). If patient specimens are diluted, they should be diluted with the same biological fluid to maintain the physiological dynamics of the system.

A graphic display or table of the known concentration vs. the observed concentration should be included in the results. The sample concentrations should be evenly distributed across the reportable range of the assay. The appropriate number of replicates and concentration levels depends on the reportable range of the assay.. "Evaluation of the Linearity of Quantitative Analytical Methods; Approved Guideline" (2003) NCCLS Document EP06-A3 describes a protocol for sample preparation, value assignment, appropriate analyte range and concentrations to test, as well as statistical design and analysis methods, and a format for statement of claims.

Some immunoassays may exhibit a "high dose hook effect," in which there is a decrease in response of the assay at high concentrations. Whenever appropriate (e.g., for two-site or sandwich immunoassays), the linearity studies should be extended beyond the reportable range to the highest concentrations that may be encountered in clinical settings in order to evaluate whether the device exhibits a high dose hook effect.

The protocol description should include sample types and preparation, concentrations, number of replicates and statistical methods used. The description of results should include the acceptable maximal differences from linearity or the measured maximal differences (including confidence intervals) from linearity and the range of linearity, as described in NCCLS EP06-A. Data from the high-dose hook evaluation, should be included

Information on how to treat samples with lamotrigine concentrations outside the reportable range should be provided. If users are recommended to dilute samples that are above the reportable range, a specific protocol for dilution, including a validation of that protocol, should be provided. It is also necessary to clarify how samples with concentrations outside the range of linearity are reported to the user.

A validated protocol recommending how to dilute patient specimens without changing the assay's performance is an essential component of every TDM assay.

#### **(4) Sensitivity**

The functional sensitivity (lower limit of quantification) of the assay is defined as the lowest lamotrigine concentration for which acceptable assay precision and accuracy are observed, and this should be characterized and reported. For lamotrigine the concentration at which the intra-assay coefficient of variation is not greater than 10% is adequate. The acceptance criteria for sensitivity of a TDM assay should take into account the expected serum/plasma concentrations at the lower limits of therapeutic dose and any possible patient non-compliance issues. The accuracy at the lower limit of quantification (LLOQ) should also be described, based on samples with known drug concentrations.

The description of the sensitivity evaluation should include sample type, definition of the measures of sensitivity and results. Clarify how lamotrigine measurements below the LLOQ are reported to the user. The sensitivity and CV may vary depending upon the sensitivity of the analytical techniques utilized.

#### **(5) Specificity for parent compound**

As a measure of assay specificity, cross-reactivity with lamotrigine metabolites should be characterised. Primary known metabolites should be included for lamotrigine specificity studies; i.e. the N-2 glucuronide, N-5 glucuronide, N-2 methyl metabolite, N-2 oxide (Sinz, 1991; Doig, 1991; Dickens, 2002). While developing a TDM assay, the developer is encouraged to establish a close working relationship with the pharmacology and drug metabolism division of the pharmaceutical manufacturer.

The description of the evaluation should include description of types of samples used for spiking, number of replicates, concentration of metabolite, computation or definition of cross-reactivity used and percent cross-reactivity for each metabolite.

Since the two lamotrigine glucuronide metabolites are pharmacologically inactive there is no requirement to determine them as part of a routine TDM assay. The N-2 methyl metabolite has some pharmacological activity but in most patients all lamotrigine metabolites are present in concentrations significantly less than those of the parent compound at any given moment in plasma or serum. However, it is important to establish the extent of metabolite cross reactivity during the development of a new lamotrigine TDM assay. A simple approach to establish whether or not lamotrigine metabolite cross reactivity is present is to spike the parent compound into a drug free plasma or serum and the same concentration into plasma/serum from patients who have taken lamotrigine. Another approach is to obtain assay results performed on patient specimens, particularly from patients with compromised renal function, and to compare such results with the results of a highly specific assay, such as mass spectrometry. It may be helpful to consult with FDA prior to undertaking this alternative type of study.

Whenever possible plasma/serum specimens from patients in renal failure who are taking lamotrigine should be retrieved and the reported concentration compared to that of specimens spiked to the same reported concentration in the same drug free serum/plasma. If the results are significantly different between the two specimens, a metabolite cross reactivity problem should be suspected. The major metabolite of many drugs is a pharmacologically inactive glucuronide which may cross react but under normal circumstances does not produce an elevated result with the TDM assay because plasma/serum concentrations are extremely low. However, patients in renal failure have extremely high glucuronide concentrations which may produce a cross reactivity with the TDM assay. This is the reason for quantitating biological fluids from patients in renal failure.

## **(6) Interference**

The effects of potential interferents on assay performance should be characterised. Potential sources of interference that you should test include, but are not limited to, the following:

Endogenous compounds, particularly those listed below; at the suggested concentrations. The object of these studies is to confirm that analyte concentrations of naturally occurring compounds that may occasionally elevated do not interfere with the TDM assay.

- bilirubin (60 mg/dL)
- triglycerides (1500 mg/dL)
- cholesterol (500 mg/dL)
- uric acid (20 mg/dL)
- rheumatoid factor (500 IU/ml)
- hematocrit (15-60%)
- albumin (12 g/dL)
- gamma globulin (12 g/dL)
- human anti-mouse antibodies, HAMA
- hemoglobin (20-2000 mg/dl, due to hemolysis)
- blood substitutes

Commonly co-administered drugs including, but not limited to those listed below.

Drugs commonly co administered to treat a specific disease should also be evaluated for potential TDM assay interferences; the list of specific drugs to be checked is dependent upon the TDM assay under development.

- all available antiepileptic drugs and relevant metabolites (see appendix 3)
- all available antipsychotic and antidepressant drugs
- Common tranquilizers and hypnotics
- commonly prescribed antibiotics
- common over-the-counter drugs

Anticoagulants or preservatives with which the sample is likely to come into contact, such as EDTA and heparin, various types of gels contained in serum separator blood collection tubes, and different collection and storage tube materials, such as plastic and glass. When testing these interferents, the concentrations of lamotrigine in the sample should be adjusted to medical decision levels. Typically, interference studies involve adding potential interferents to the sample containing the drug and determining any bias in recovery of lamotrigine, relative to a control sample (to which no interferents have been added). In addition to anticoagulants and endogenous substances it is essential that the various specialized biological fluid collection devices e.g. serum gel separators, filter paper, and ultra filtration membranes also be evaluated.

Recommended guidelines for interference testing are described in detail in “Interference Testing in Clinical Chemistry; Approved Guideline” (2002) NCCLS Document EP07-A4. This document includes guidelines for setting decision criteria as well as for protocol designs, statistical methods, evaluating interference using patient specimens and establishing validating and verifying interference claims. The following considerations should be included when interferent testing is being planned:

- For endogenous substances, test at the highest concentration expected based on experience with the intended use population. Interference studies using samples naturally high in the endogenous compound being tested can be informative and this approach should be considered when such samples are available.
- For drugs, test to levels 3 times the highest acute peak concentration reported following therapeutic dosage.
- For specimen additives, test up to levels five times the recommended concentration.

If interference is observed at the concentration levels tested, lower levels should be tested in order to determine the lowest concentration that could cause interference. Replicate samples should be tested in these protocols. The description of the evaluation should include the following items (if description of the protocol refers to NCCLS EP07-A, clarify which aspects of the guidelines were followed):

- names and concentrations of interferents tested
- sample type (e.g., spiked whole blood pools, samples naturally high in endogenous compounds)
- concentrations of target drug in the sample
- number of replicates tested
- definition or method of computing interference.

When reporting results, any observed trends in bias (i.e., negative or positive) across the concentration range of interferents tested must be identified. Include the standard error of the observed recoveries at each concentration or the range of observed recoveries at each concentration evaluated for a potential interferent. This approach is more informative than listing average recoveries alone.

For substances listed as non-interfering, state the criteria on which this is based, e.g., inaccuracies due to these substances are less than 10% at a given lamotrigine concentration. If any compounds are known from the literature or other sources to interfere with the test system, these should be included among the information in the labeling. It may not be necessary to perform additional interference testing with these known interferents.

#### **(7) Specimen collection and handling conditions**

The labeled recommendations for specimen storage and transport must be substantiated, by assessing whether the device can maintain acceptable performance (e.g., precision and accuracy) over the storage times and temperatures (including freeze/thaw cycles), recommended as acceptable by the manufacturer. An appropriate study includes analysis of sample aliquots stored under the conditions of time, temperature, or allowed number of freeze/thaw cycles recommended in the package insert. Storage conditions and freeze-thaw cycles are especially important for research studies where long specimen storage periods are required. Manufacturers' should update the package insert as new information on storage criteria becomes available.

#### **(8) Method comparison**

The new lamotrigine assay must be compared with a reference method, specific for the parent compound. Fully validated chromatographic methods that specifically measure parent drug should be used as the comparator in such a study (Berry, 1992; Fraser, 1995; George, 1995; Forssblad, 1996; Lensmeyer, 1997; Croci, 2001). If the discordance exceeds 10% relative to the reference procedure, the reasons for the discordance should be addressed. It behoves the TDM assay developer to compare their new assay to any analytical technique that may be routinely utilized in clinical chemistry laboratories for drug analysis particularly if simpler LC, GC, immunoassay or other techniques are published or available. Such initial comparisons allow the manufacturer to establish the performance of the TDM assay under various analytical conditions.

Guidelines provided in the document, "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline" (1995) National Committee for Clinical Laboratory Standards, Document EP09-A5 concerning experimental guidelines and statement of claims should be followed. Epileptic patient samples with lamotrigine concentrations distributed across the reportable range of the assay, should be evaluated. Banked (retrospective) samples are appropriate for these studies as long as the information on the sample population is available to characterize the specimens. Samples from multiple geographic sites or clinical centers should be included. In general pre-dose blood is the preferred sample for TDM of lamotrigine, but for the

purpose of a method comparison, any time of sampling would be acceptable. Factors such as age and time of blood draw with respect to drug administration (e.g., trough, peak) should be noted since these might influence drug-to-metabolite ratios and consequently, assay bias (although significant concentrations of the major lamotrigine metabolites will not be expected to accumulate in most patients to cause bias). Ideally sufficient clinical information should be collected to enable Concentration Dose Response Ratios to be calculated.

Appropriate sample size depends on factors such as precision, interference, range, and other performance characteristics of the test. The number of patients should also be large enough so that inter-individual variation would be observed. A statistical justification to support the study sample size should be provided in the protocol description. It is expected that the sample size target, however supported, will include a minimum of 100 samples distributed fairly evenly over a minimum of 50 *individual patients*.

If multiple measurements from individual patients are included, the results should be summarized using appropriate statistical analyses such as Analysis of Variance, Generalized Estimating Equations, or Bootstrapping, to account for correlation of repeat measurements within patients in the study.

For the results to be properly interpreted all relevant information on the sample population should be provided in the package insert. Information on the sample population should include:

- the number of individual patients represented by the samples
- the number of data points
- the number of clinical sites
- information regarding the time of last dose

Any specific selection (inclusion or exclusion) criteria for samples should be stated together with an indication of whether samples were collected from patients with specific clinical outcomes, or from centers using atypical or novel drug regimens. Factors such as age range (e.g., adults), and time of blood draw with respect to drug administration (e.g., trough, peak) might influence drug-to-metabolite ratios and consequently, assay bias (although significant concentrations of the major metabolites would not be expected to accumulate sufficiently in most patients to cause bias). In general, pre-dose blood is the preferred sample for TDM, but for the purpose of a method comparison, any time of sampling would be acceptable.

Ideally one would like sufficient clinical information to be able to calculate Concentration Dose Response Ratios. Often, however, it is not practical to obtain dosing information and sample draw times for stored laboratory specimens. Storage conditions can affect the quantitation of specimens particularly if they have been stored for an extended period of time. If there is wide variance between the TDM assay and

reference method in stored specimens, it is suggested that the specimens be re-assayed utilizing the reference technique before comparing results with the new TDM assay. Such analysis compensates for storage changes that alter drug concentrations.

The comparator methods used must be clarified, and references to validation of the procedure included. If samples evaluated in the study include both trough and other times of blood draw relative to drug administration, a separate statistical analysis for these groups should be conducted. When providing the results of the method comparison study, the following information should be included:

Scatterplots of the new assay versus the reference method. The plots should contain all data points, the estimated regression line and the line of identity. Data points in the plot should represent individual measurements.

A description of the method used to fit the regression line and results of regression analysis including the slope and intercept with their 95% confidence limits, the standard error of the estimate (calculated in the y direction), and correlation coefficient should be included. In cases where parameters are not consistent throughout the reportable range, estimates of more than a single range may be appropriate. If the comparator, as well as the new assay is subject to measurement error, a regression method such as the Deming method may be appropriate, rather than Least Squares.

- To illustrate the degree of inter-individual variations, include graphs of difference in measurements (i.e., new device minus reference HPLC method) versus the reference HPLC method. Appropriate representations include a bias plot of difference in measurements ( $y - x$ ) versus the reference method ( $x$ ), as recommended in NCCLS Document EP09-A, or versus the mean of  $y$  and  $x$ , as recommended by Bland and Altman (Bland, 1995).

The points above apply to any reference method. The more information that is available comparing the reference method to the new lamotrigine TDM assay, the easier it is for the reviewer to recognize the validity of the new assay. Providing the information initially in sufficient detail and clarity speeds the review process and we emphasize the importance of clear and frequent communication with the FDA Diagnostics Division during the development of any new TDM assay.

A variety of clinical circumstances can influence the interpretation of any drug concentration. The purpose of a TDM assay is to provide a tool that can be utilized in conjunction with other clinical parameters and diagnostic procedures to enhance any clinician's ability to provide optimal patient care through the use of Therapeutic Drug Management at any time. More efficient and better the patient's care will result from more readily available TDM assays for the newer AEDs.

#### **(9) Studies at external sites**

Performance of the lamotrigine assay should be evaluated in at least 3 external laboratory sites in addition to that of the manufacturer's site. This may be included as

part of the method comparison study described above. Data from individual sites should initially be analyzed separately to evaluate any inter-site variation. Method comparison results from the individual sites can be pooled in the package insert, if it is demonstrated that there are no significant differences in results among sites.

#### **(10) Calibrators**

Provide the following information about the calibrators in the assay kit in the summary report:

- Protocol and acceptance criteria for real-time or accelerated stability studies for opened and unopened calibrators.
- Protocol and acceptance criteria for value assignment and validation, including any specific instrument applications or statistical analyses used.
- Identification of traceability to a domestic or international standard reference material.
- Protocol and acceptance criteria for the transfer of performance of a primary calibrator to a secondary calibrator.

For information about calibrators marketed separately as class II devices under 862.1150, see the guidance "Abbreviated 510k Submissions for *In Vitro* Diagnostic Calibrators,"

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## 6. Appendices

### Appendix 1

#### Metabolism and disposition

Optimal serum/plasma concentrations for individual patients depend upon many factors such as patient pharmacogenomics and tolerance of the drug, co-administered drugs, and co-pathology. In healthy volunteers approximately 10% of lamotrigine is excreted unchanged in the urine and the remainder is extensively metabolized, primarily to a pharmacologically inactive N-2 glucuronide (70%) and N-5 glucuronide (10%) with lesser quantities of N-2 methyl metabolite, N-2 oxide and some as yet unidentified compounds (Doig, 1991; Sinz, 1991). Glucuronidation is a major conjugation pathway that is catalysed by a number of different isoforms of UDP-glucuronyltransferase (UGT). N-glucuronidation is a well-established route in the human metabolism of drugs containing a tertiary amine group (Hawes, 1998). Substrate specificity of many UGTs is unclear, but UGT1A4 has been implicated in the formation of quaternary amine linked glucuronides including lamotrigine (Green, 1995). Lamotrigine has also been cited as a UGT1A4 substrate in inhibition experiments with the developmental anticonvulsant, retigabine (Hiller, 1999). All the evidence indicates that lamotrigine N-2 glucuronidation, the major route of metabolism in humans, is catalysed by UGT1A4. This pathway is inhibited by valproate and induced by hepatic enzyme stimulating anticonvulsants (see earlier refs.). However, patients on monotherapy with lamotrigine are reported to display large variation in the dose to serum level relationship (Wertz, 1999) and this may be due to genetic polymorphism in the UGT1A4 drug metabolising enzyme. Despite these many factors that might influence the clearance of lamotrigine, it is not necessary to undertake pharmacogenetic testing of an individual prior to placing them on this drug. Dosage adjustments with guidance from TDM are adequate.

While these glucuronide and other metabolites are normally cleared rapidly from plasma; all immunoassays should ideally be developed to have minimal cross reactivity with metabolites. In addition, performance observed for a new assay relative to a gold standard analytical technique (e.g. measures of bias, variability, cross-reactivity) should be clearly portrayed by the manufacturer in the labeling. If this drug is approved for non-epilepsy applications, the recommended optimal concentration range may be different from that necessary to maintain seizure control.

## Appendix 2

Identified Risk	Recommended Mitigation Measures
Analytical error overestimating lamotrigine concentration	Documented accuracy and analytical specificity throughout the measurement range
Analytical error underestimating lamotrigine concentration	Documented accuracy throughout the measurement range
Analytical imprecision in estimating lamotrigine concentration	Documented precision throughout the measurement range
Analytical interference resulting in substances other than lamotrigine being measured and reported	Documented cross-reactivity of substances other than Lamotrigine

## Appendix 3

Interference evaluation/validation	Recommendation
Antiepileptic drugs	<ul style="list-style-type: none"> <li>• Acetazolamide</li> <li>• Carbamazepine</li> <li>• Carbamazepine-10,11-Epoxyde</li> <li>• Clobazam</li> <li>• Clonazepam</li> <li>• Desmethyloclobazam</li> <li>• Desmethylnethsuximide</li> <li>• Diazepam</li> <li>• Ethosuximide</li> <li>• Ethotoin</li> <li>• 5-ethyl-5-phenylhydantoin</li> <li>• Felbamate</li> <li>• Gabapentin</li> <li>• Mephentyoin</li> <li>• Methsuximide</li> <li>• Levetiracetam</li> <li>• Nitrazepam</li> <li>• Oxcarbazepine</li> <li>• 10-hydroxycarbamazepine</li> <li>• 2-phenyl-2-ethyl-malonamide (PEMA)</li> </ul>

	<ul style="list-style-type: none"><li>• Phenobarbital</li><li>• p-hydroxyphenobarbital</li><li>• p-hydroxyphenylhydantoin glucuronide</li><li>• Primidone</li><li>• Stiripentol</li><li>• Tiagabine</li><li>• Topiramate</li><li>• Valproic acid</li><li>• Vigabatrin</li><li>• 3-keto-Valproic acid</li><li>• Zonisamide</li></ul>
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